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1. Donnelly, J.J., Ulmer, J.B., Liu, M.A. Minireview: DNA vaccines. Life Sciences vol. 60 no. 3, pp 163-172 (1997)

2. Ulmer, J.B., Sadoff, J.C., and Liu, M.A. DNA vaccines. Current Opinion in Immunology vol. 8, no. 4, pp 531-536 (1996)

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MINIREVIEW

DNA VACCINES

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Summary

Immunization with plasmid DNA encoding antigenic proteins elicits both antibody and cell-mediated immune responses. This method of producing the protein antigens of interest directly in host cells can provide appropriate tertiary structure for the induction of conformationally specific antibodies, and also facilitates the induction of cellular immune responses. DNA immunization has provided effective protective immunity in various animal models. The immune responses induced by DNA vaccines may in some instances be preferable to those produced by immunization using conventional methods. DNA vaccination appears to be applicable to a variety of pathogens and is a useful method of raising immune responses. Thus this approach to vaccination has the potential to be a successful method of rapidly screening for antigens capable of inducing protective immunity, and of inducing protective immunity against pathogens of clinical importance.

Key Words: immunization, DNA, nucleic acids, intramuscular, intradermal, biolistic, needleless, protective immunity, influenza

For vaccine technologies, success can be measured in the number of cases of clinically apparent disease prevented and the extent to which the technology is employed in human populations. A successful vaccine technology must satisfy several requirements. The technology must elicit an appropriate immune response for prevention of mortality and morbidity in individuals. Since not all members of the target population will be vaccinated, and since not all those vaccinated will be protected, the protective immune responses induced should be able to limit transmission by vaccinees to help protect the population as a whole. Furthermore, protection should persist in the absence of transmission, to prevent re-entry of the pathogen into the target population. The technology must be very safe, so that mass immunization of healthy imdividuals can be carried out without intensive followup. The technology also should have the flexibility to present a number of distinct antigens from a given pathogen, to guard against the emergence of novel variants or of escape mutants and to provide efficacy across a broad spectrum of human genetic backgrounds.

Finally, the technology should be adaptable for use against many target pathogens. Examples of technologies that have provided efficacious vaccines include live attenuated viruses (smallpox, polio, measles, mumps, rubella, varicella), whole killed bacteria (*B. pertussis*, *V. cholerae*), inactivated viruses (influenza A, polio), and subunits or fragments of the causative agent (hepatitis B surface antigen).

DNA vaccination has been studied with increasing intensity as a potentially successful vaccine technology for the future ever since the initial demonstrations that direct transfection in vivo with DNA could be used to express foreign proteins and thereby induce protective immune responses. A number of factors contribute to the appeal of DNA vaccines. Preclinical studies performed so far have indicated that the antigenicity of viral proteins produced by DNA vaccination is very similar to that of the proteins when produced naturally during infection. In addition, immunization by direct transfection in vivo with plasmid DNA is an efficient means of eliciting $CD8^+$ cytotoxic T lymphocytes (CTL). These CTL recognize peptides 9-11 amino acids in length bound to Class I molecules of the Major Histocompatibility Complex (MHC). Thus, protein antigens produced by DNA vaccination gain access to pathways of antigen presentation via Class I MHC molecules, a system of antigen presentation that is most frequently utilized by live attenuated virus vaccines, recombinant viral vectors, and intracellular bacteria such as *Listeria* or *Shigella*. This mechanism of antigen presentation is not generally utilized by recombinant protein vaccines or by inactivated virus vaccines. DNA vaccines therefore could serve as alternatives to live virus vaccines where use of an attenuated agent would be of concern, as in the case of HIV. Experience in laboratory animal models has indicated that multiple biochemically similar antigens from different strains of the same pathogen, or multiple distinct proteins of a pathogen, can in some instances be combined in a single vaccine by the simple expedient of mixing together multiple plasmid DNAs. The relative simplicity of construction of vectors for DNA vaccination and the generic purification technology for plasmid DNA should increase the speed and decrease the effort required to develop novel vaccines, providing a tool for the rapid screening of potential protein immunogens, and making effective vaccines more widely available. In the future, the flexibility of this technology may allow antigens expressed in DNA vaccines to be "engineered" to improve processing, extend the diversity of epitopes presented, or increase levels of expression. In addition the antigens might be coexpressed with cytokines. The promise of the technology, however, remains to be judged on its ability to produce protective immunity in the target human and animal populations.

Intramuscular DNA Vaccines against Infectious Diseases

Humoral and Cellular Immune Responses

Both antibodies and cell-mediated immune responses have been induced by direct intramuscular inoculation with plasmid DNA encoding viral proteins. These responses have been found to be protective in various animal models of viral diseases. Antibody responses were first demonstrated for an influenza virus protein in mice and nonhuman primates (1), as well as for other viral proteins in mice, cattle, and nonhuman primates (2-10). Plasmid DNAs encoding the hemagglutinin (HA), matrix protein, and nucleoprotein (NP) from influenza A virus (1-3), gp120 (4, 5), and gp160 (6, 7) from HIV, gIV from bovine herpesvirus I (8), rabies virus surface glycoprotein (9), hepatitis B surface antigen (10, 11), hepatitis C core protein (12, 13), hepatitis D virus (14), NP of lymphocytic choriomeningitis virus (LCMV) (15, 16), major capsid protein of cottontail rabbit papilloma virus (CRPV) (17), and gB (18) and gD (19) of herpes simplex virus (HSV) were shown to induce antibodies after intramuscular injection. In some instances the plasmid DNA was administered directly in saline solution (1-5, 8, 9, 13-19), while in other studies the DNA was administered after injection of a toxin or local anesthetic intended to cause necrosis and regeneration of the injected muscle to increase expression of the gene of interest (6,7,10-12, 20). Virus

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neutralizing antibodies were raised against influenza HA (2), gp160 of HIV (6, 7, 20), surface glycoprotein of rabies virus (9), CRPV (17) and HSV (18). The ability to generate hemagglutination inhibiting (HI) and neutralizing antibodies indicates that the influenza HA is present in its native conformation in, or on the surface of, the transfected cells, since the epitopes of influenza HA that are recognized by these antibodies are formed by noncontiguous regions of the HA polypeptide and since cell surface expression of HA further requires that it be assembled into trimers (20). Durable antibody responses (4 months or more) were obtained by intramuscular injection of influenza HA DNA in African green monkeys (22) and in mice (20 months or more; Ulmer et al., unpublished observations). Further re-immunization of African green monkeys with plasmid DNA encoding influenza HA one year after the initial immunization induced substantially increased booster HI antibody responses (Donnelly et al., unpublished observations).

DNA vaccines also have been used to raise immune responses against antigens of nonviral pathogens. Antibodies have been raised successfully against proteins from a metazoan parasite, *Schistosoma japonicum* (23), two protozoans, *Leishmania major* (24, 25) and *Plasmodium yoelii* (26-28), two bacteria, *Mycobacterium tuberculosis* (29, 30) and *Salmonella typhi* (31), and *Mycoplasma pulmonis* (32), by intramuscular injection of plasmid DNA. Protective immune responses also have been demonstrated in mice immunized against *L. major*, *P. yoelii*, *M. tuberculosis* and *M. pulmonis* using plasmid DNA (24-28, 30). The types of protective responses elicited by the DNA vaccines are not yet well characterized in these models, but in the case of *L. major*, *P. yoelii*, and *M. tuberculosis*, protection is likely to be cell-mediated in nature. Immunization of BALB/c mice with a plasmid encoding gp63 of *L. major* induced a helper T cell response with a dominant Th1 phenotype. BALB/c mice are normally susceptible to *L. major* infection due to an inability to mount a Th1 response, unless given exogenous IL-12 (33), suggesting that DNA vaccination also may provide a means to overcome this deficit.

As little as 1 μg of HA DNA from A/PR/8/34 (H1N1), injected intramuscularly 2 times, completely protected mice from disease following a lethal homologous challenge (34). Intramuscular immunization of mice with plasmid DNA encoding the rabies virus glycoprotein protected them from death following challenge with 5 LD_{50} of rabies virus (9). Ferrets immunized with HA DNA from the recent clinical isolate A/Georgia/03/93 (H3N2) or the A/PR/8/34 strain had significantly reduced nasal viral shedding when challenged with the corresponding homologous influenza strain (22). In cattle (the target population for bovine herpesvirus immunization), intramuscular injection of DNA coding for the gIV of bovine herpesvirus likewise protected against challenge (8), while injection of DNA encoding HSV-2 gB or gD protected mice and guinea pigs from challenge with HSV (18, 19, 35).

Cottontail rabbit papilloma virus (CRPV) illustrates the ability of intramuscularly injected plasmid DNA to elicit conformationally specific, virus neutralizing antibodies against encoded proteins and concomitant protective immunity. Immunization with the major capsid protein, L1, of CRPV induces neutralizing antibody responses when the L1 is assembled in its native form, for example in CRPV-infected rabbits or rabbits immunized with virus-like particles (VLP) (36). In contrast, denatured L1 does not induce virus neutralizing antibodies (36). The processing of CRPV L1 may differ substantially from that of other viral surface proteins studied previously using DNA vaccines, such as influenza HA(2), hepatitis B surface antigen (10) and HIV gp160(6, 7), since papillomavirus virions assemble in the nucleus of infected epithelial cells (36) rather than at the cell surface. Thus, CRPV L1 might not normally be secreted by a classical secretory mechanism nor expressed at the cell surface. However, both virus-neutralizing antibodies and ELISA activity against intact L1 VLPs were detected in NZW rabbits injected with plasmid DNA encoding L1 (17). Rabbits immunized with L1 DNA also were protected from the development of warts upon challenge with virulent CRPV. Thus the ability of DNA immunization to elicit antibodies recog-

nizing viral proteins in their native conformations is not limited to proteins targeted for secretion or expression on the host cell membrane.

Cytotoxic T lymphocytes (CTL), can be demonstrated readily when spleen or lymph node cells from mice injected intramuscularly with plasmid DNA encoding viral antigens are restimulated in vitro with antigen, or with mitogen and IL-2, or, in the case of LCMV, restimulated in vivo by viral infection. Effector CTL that recognize peptide epitopes appropriate to the H-2 restriction element have been demonstrated in mice immunized with DNA encoding the NP from influenza A virus (1), hepatitis B surface antigen (37), and HIV *env* (38, 39, 40), and in nonhuman primates imunized with DNA encoding HIV *env* (41). CTL capable of recognizing and killing virus-infected targets were demonstrated by Ulmer et al (1) against influenza virus, by Wang et al. (42) using targets infected with vaccinia-HIVgp160 recombinants, by Xiang et al (9, 43) using vaccinia virus or adenovirus-rabies virus glycoprotein recombinants, and by Yokoyama et al (15) and Pedroza-Martins et al (16) using LCMV. In studies of influenza NP in mice, a single intramuscular injection of as little as 1 μg of NP DNA (34) induced CTL against influenza NP. In other studies, anti-NP CTL were found to persist for more than two years after immunization with influenza NP DNA (44, 45).

Protection in Preclinical Models

Cell-mediated protection has been demonstrated in mouse models using both influenza and LCMV. The protective efficacy of a DNA vaccine was first demonstrated using influenza virus; mice that had been injected intramuscularly with NP DNA were protected from lethal virus challenge (1). The NP DNA immunization provided cross-strain protection, in that the immunizing NP gene was cloned from an influenza strain that, in addition to being of a different subtype, had been isolated 34 years before the challenge strain (1, 34). The protection observed in mouse models of influenza was found to be a result of cellular immunity against NP, since passive transfer of serum from mice immunized with NP DNA did not protect although the serum contained high titers of antibody against NP (1). In contrast, adoptive transfer of splenocytes from mice immunized with NP DNA (restimulated in vitro prior to transfer according to methods described in ref. 46) did provide protection against lethal challenge (T.-M. Fu and J.J. Donnelly, unpublished observations). In mice that were actively immunized in vivo with NP DNA, depletion of $CD4^+$ T cells in vivo prior to lethal challenge with influenza virus reduced the extent of protection while depletion of $CD8^+$ T cells abrogated protection completely, indicating that both $CD8^+$ and $CD4^+$ T cells contributed to the protection (J.B. Ulmer and M.J. Caulfield, unpublished observations). In contrast to the protective efficacy seen in these studies where DNA immunization resulted in the endogenous production of the antigen, immunization with purified NP as protein rather than as DNA did not induce a CTL response, nor did it confer any protection (47). Yokoyama et al (15) found that both BALB/c and C57BL/6 (B6) mice immunized with DNA encoding the NP of LCMV were protected from lethal challenge, while only B6 mice could be protected by immunization with glycoprotein (GP) DNA. LCMV NP contains CTL epitopes that can be recognized by both BALB/c and B6 mice, while LCMV GP contains epitopes that can be recognized by CTL of B6 mice but lacks CTL epitopes that can be recognized by BALB/c mice (15). This observation suggests that DNA-induced CTL contributed significantly to the protective effect of GP DNA against LCMV in B6 mice.

Influenza virus infection in the ferret provides a model that resembles the pathophysiology of human influenza more closely than that found in the mouse. In ferrets influenza infection is localized to the nasal turbinates and trachea, and the progression of the disease can be monitored by determining the level of virus shed in nasal washes, as is done in human studies of influenza vaccines. Ferrets are highly susceptible to infection with human isolates of influenza virus, allowing clinical isolates to be studied directly without host adaptation. Immunization of ferrets with HA

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NA elicited hemagglutination inhibiting (HI) antibody responses comparable to those induced by mmercial inactivated virus vaccine, and provided significant reduction in virus shedding if nimals were challenged with the homologous strain of influenza (22). When ferrets were immuzed with HA DNA or the currently licensed inactivated virus vaccine but challenged with a fferent strain of influenza virus, e.g., one that had undergone antigenic drift and thus differed om the immunizing strain by 17 point mutations in the HA1 region of the HA molecule, virus edding was not reduced until day 6 after infection. A corresponding phenomenon can occur in uman populations when an influenza strain undergoes antigenic drift during an influenza season; gnificant deviation of the epidemic strain from the vaccine strain results in disease even in highly accinated populations. When ferrets were immunized intramuscularly with NP DNA, the animals ere able to be infected with influenza virus and shed virus, but experienced significantly reduced ral shedding in nasal washes 5-6 days after challenge (22 and Donnelly et al., unpublished obervations). This immunity was cross-strain in that similar reductions of shedding were obtained hen the challenge strain was a drift variant, or even a different subtype, from the immunizing rain. Combined immunization of ferrets with plasmids encoding the NP, matrix (M1) and HA enes provided protection against an antigenic drift variant, equivalent to the protection achieved immunization with homologous HA DNA (22). These results illustrate the potential utility of mmunization with plasmid DNA encoding multiple proteins from influenza, both to generate otective antibody responses and to induce cellular immunity that is able to confer cross-strain otection.

Alternatives to Intramuscular Injection

Intradermal Injection

lternative methods for the delivery of DNA vaccines have been evaluated in a number of labotories in several forms. Intradermal administration of plasmid DNA using conventional needle jection methods was investigated in our laboratory and elsewhere (48-51). Results using intradrmal administration varied with both the antigen expressed and the species studied. In the case of fluenza NP, although both antibody and CTL responses were induced in mice by this method 48, 49), protection studies in our laboratory comparing mice immunized intramuscularly and tradermally with NP DNA suggested that the intradermal method was less effective (49). Studies ing rabies glycoprotein in mice showed that immunization by both routes induced antibodies 51). In studies using plasmids encoding three different malaria genes, DNA encoding a hepatic nd erythrocytic stage antigen (HEP17) protected mice when given by either route while DNA ncoding circumsporozoite protein (CSP) or sporozoite surface protein 2 (SSP2) gave better proection by the i.m. route (50). Conversely, antibody responses in mice to SSP2 were greatest by e i.d. route while the i.m. route gave greater antibody responses for CSP and HEP17 (50). Plasid DNA encoding CSP induced antibody responses in *Aotus* monkeys only when given by the i. route (50). DNA encoding HIV *env* induced antibodies in Rhesus monkeys only when given the i.m. route (J.W. Shiver, personal communication). Thus neither route is conclusively fared over the other in all instances.

Particle-mediated Transfection

ng et al (52) applied in mice a technique which they originally developed for tranfection of ant cells, termed "biolistic immunization." In this method DNA is coated onto colloidal gold rticles which are then fired into the cells of interest using a chemical propellant, expansion of ter vapor, or expansion of compressed gas as the energy source (53). The original description Tang et al (52) used the technique to introduce the gene for human growth hormone into the idermis of mice. Keratinocytes and some dermal fibroblasts became transfected and produced

sufficiently high levels of human growth hormone to allow its detection in the serum using an assay specific for the human protein. The mice also developed serum IgG antibodies to human growth hormone. Subsequently Fynan et al. (54) applied the biolistic transfection technique to a mouse influenza model. Delivery to the epidermis and dermis of DNA encoding the HA from A/PR/8/34 driven by a CMV IE promoter primed the mice for an anamnestic response to influenza infection and protected the mice from a subsequent lethal challenge with the homologous strain of influenza. Low levels of serum HI antibodies (titer of 1:10) to A/PR/8/34 were detectable in the mice prior to the influenza virus challenge; substantially higher HI antibody titers were present in surviving mice after challenge indicating that the level of immunity produced was not sufficient to completely prevent infection. (Immunization by intramuscular injection of DNA encoding the HA from A/PR/8/34 induced high levels of HI anitbody before challenge, and no rise in HI titer was observed after virus challenge, indicating a more complete suppression of viral replication (2; J. Ulmer, unpublished observations).) Webster et al (55) studied biolistic immunization in a ferret model using the same influenza strain, A/PR/8/34. In this study viral shedding was used as a measure of immunity, and biolistic immunization with HA DNA was found to eliminate detectable viral shedding on days 3 and 5 after infection. However some virus replication was allowed since all animals showed substantial increases in serum neutralizing antibody titers after challenge (55). (In ferrets immunized by the i.m. route with DNA encoding HA from A/PR/8/34, some individuals had not only no detectable virus shedding but also no significant rise in HI antobdy titer after challenge, suggesting a more complete suppression of virus replication (22; A. Friedman and J.J. Donnelly, unpublished observations).) The amount of DNA delivered by biolistic immunization in the mouse and ferret studies was approximately 0.4 mcg per "shot." Increased efficacy was obtained when repeated "shots" were given to deliver a total quantity of DNA of approxmately 2 μg.

Induction of cell-mediated immunity after biolistic transfection was first demonstrated directly by Hui et al (56) using the gene for the MHC antigen H-2K^b administered intramuscularly or intrasplenically after surgical exposure of the target tissues. Mice immunized by this route developed allospecific CTL. A combined approach of intramuscular injection followed by biolistic inoculation was used to raise CTL recognizing a V3 loop peptide of HIV as well as virus neutralizing antibodies and antibodies to HIV p24 in mice (57). Although antibody responses to p24 that had been induced by intramuscular injection of DNA were increased by boosting by the biolistic method, anti-*env* antibody responses declined after the second i.m. injection and continued to decline even when biolistic booster immunizations were given (57). CTL responses detected in splenocytes restimulated in vitro with the V3 loop peptide persisted to 15 weeks after the final immunization although the *env* antibody responses had declined to undetectable levels by this point (57). In separate studies using biolistic immunization exclusively, mice given 3 biolistic immunizations of 4 μg each of a construct encoding gp120 and 1 μg each of a construct encoding *rev*, CTL responses were induced after 2 immunizations but were suppressed by a third immunization, while antibody responses appeared only after dose 3 (58). The authors suggested that the suppression was related to a switch in helper T cell phenotypes from Th1 to Th2, as the loss of CTL responsiveness was blocked by administration of antibody to IL-4 (58). (These observations differ from studies using i.m. immunization where repeated immunization and boosting increased cell-mediated immune responses to influenza NP (47) and HIV *env* (5, 40), and have been reported to drive immune responses toward a Th1 phenotype (28).) Studies comparing the induction of CTL using an influenza NP construct administered biolistically or by intradermal injection indicated that, for this particular construct, injection of 1 μg of DNA i.d. with a conventional needle did not induce CTL whereas as little as 16 ng of DNA did induce CTL by gene gun immunization (59). Comparison of antibody responses induced by intramuscular and gene gun immunization of mice with a construct encoding human alanine amino-tranferase indicated that higher antibody titers were induced after administration of 16 ng of DNA by the gene gun than were

nduced by 1 μg of DNA given intramuscularly (59). However in these studies the i.m. injections vere given into surgically exposed quadriceps muscles, a technique that in our laboratory was found to give poorer protective immune responses than closed i.m. injection through intact skin (J.J. Donnelly et al., unpublished observations).

Most recently, biolistic immunization has been used with genes encoding fusion proteins incorporating an expression library from *Mycoplasma pulmonis* as a means of providing protective immunity and of screening candidate antigens (60). Serum antibodies were induced against both the human growth hormone moiety and the coexpressed *M. pulmonis* proteins. Cell-mediated immune responses also were demonstrated in vivo (footpad swelling response) and in vitro (macrophage migration inhibition). When immunized mice were challenged with *M. pulmonis*, recovery of organisms from the lungs was reduced by 4 orders of magnitude at a low challenge inoculum (10^3 CFU) and 2 orders of magnitude at a high challenge dose (10^6 CFU). Similar responses were obtained when the plasmids encoding *M. pulmonis* DNA were injected intramuscularly by conventional means (32).

Biolistic immunization also has been used in studies of resistance to LCMV infection and disease in mice (61). Immunization of mice with a DNA expression plasmid encoding the NP of LCMV driven by a CMV IE promoter/enhancer induced low levels of CTL precursors in mice given a single inoculation. Repeated immunizations increased the frequency of precursor CTL but the resulting increase only reached a level 1/100 as high as that induced by LCMV infection. Serum antibodies against LCMV NP were not detected. Repeatedly immunized mice were able to reduce viral titers in the spleen by 3 orders of magnitude after intraperitoneal challenge but were only marginally protected (30% survival) from a 100% lethal intracranial LCMV challenge. (In separate studies, both BALB/c and C57BL/6 (B6) mice immunized intramuscularly by injection of plasmid DNA encoding the NP of LCMV, and B6 mice similarly immunized with LCMV GP DNA, were protected from lethal challenge with LCMV(15, 16).)

"Needleless" Injection

Immunization with DNA also has been attempted by investigators using "needleless" injection apparatus. These devices use a piston driven by compressed gas to deliver a thin stream of inoculum under high pressure. This stream is capable of penetrating tissue and has been used clinically for the intramuscular delivery of conventional vaccines as well as for the subcutaneous administration of pharmaceuticals such as insulin (62). Vahlsing et al. (63) detected reporter gene expression, albeit at levels tenfold less than those observed after conventional intramuscular injection, as well as both antibodies and CTL to influenza NP after immunization of mice with a needleless injector. However in studies in vitro they observed significant shearing of DNA after expulsion from the device, with an increase in the proportion of linear DNA as well as the detection of smaller fragments. Furth et al. (64, 65) obtained reporter gene expression in mammary glands of lactating ewes and pregnant mice after jet administration of DNA. However in approximately 1/3 of instances this procedure proved fatal for the mice due to penetration of the injection stream through the gland and underlying muscles into the peritoneum (64). Studies in our laboratory using needleless administration of trypan blue dye to rabbit triceps femoris muscles indicated that near the skin surface the fluid stream penetrated cleanly through the muscle, while in deeper tissues the dye distribution followed connective tisssue planes resulting in extensive staining of fascia and minimal staining of muscle fiber bundles (J.J. Donnelly, unpublished observations).

Potential Clinical Uses

DNA vaccination offers the potential for the development of clinically useful vaccines for a number of infectious diseases. The use of plasmid DNA for vaccination allows both cell-mediated and humoral immune responses to be generated concurrently; to date, this has been difficult to accomplish with nonreplicating vaccines. Viral, bacterial, or parasitic diseases in which CMI is thought to have an important role in host resistance, or in which antibody responses to proteins are protective but the protein antigens themselves are not readily available are among the most immediate candidates for a DNA vaccine approach. Besides infectious diseases, cancer immunotherapy may employ polynucleotide vaccines because the simplicity of the technology allows vaccines to be tailored to individual patients (66, 67). DNA vaccines also may generate alloimmune responses capable of destroying tumor cells (68, 69). The safety and efficacy of DNA vaccines in humans are unproven as yet. Aspects of this technology, such as concerns over the potential for the induction of tolerance, immune responses against the injected DNA, and integration of the injected DNA, will require careful preclinical and clinical study. However, the broad potential utility of DNA vaccination has been shown by successful efficacy studies in animal models against viral, bacterial, and protozoan pathogens and in tumor models.

Conclusion

Polynucleotide immunization with plasmid DNA elicits both antibody and cell-mediated immune responses, and is simple and robust. Producing the protein antigens of interest directly in host cells has been shown to provide appropriate tertiary structure for proper antigenicity as well as to facilitate the induction of cellular immune responses. In various animal models in which challenge studies have been performed, this method of immunization has provided effective protective immunity. The immune responses induced may in some instances be preferable to those produced by immunization using conventional methods. Conventional methods of immunization with protein antigens may not induce reliable CTL responses, may not present proteins in appropriate conformations, and may complicate the administration of combination vaccines, all of which have been accomplished readily in preclinical animal models using DNA vaccines. DNA vaccination appears to be applicable to a wide variety of pathogens and is a useful method of raising immune responses. Thus this approach to vaccination may prove to be a successful method of rapidly screening for antigens capable of inducing protective immunity, and of inducing protective immunity against many pathogens of clinical importance.

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